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Evaluating the protective role and phytotoxic effect of *Ficus virens* in wistar albino rats

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Abstract

The methanolic leaf extract of *Ficus virens* was evaluated for protective role and phytotoxic effect in Wistar albino rats. Preliminary phytochemical analysis was carried out by using HPTLC. For studying the protective role, rats divided into G1, G2, G3 as normal, CCl₄, standard (Vit-C @ 200 mg/kg) control and G4, G5, G6 as test groups treated with extract 50,100 and 150 mg/kg BW respectively for 7 days. Oxidative stress has been induced by CCl₄ (1:1 liquid paraffin @ 1ml/kg) after 6 h of the last treatment to all animals of G2 to G6. On day 8th blood samples and liver tissue processed for different antioxidant enzyme activity including lipid peroxidation. For phytotoxic study, repeated dose 28-day oral toxicity was carried at limit dose of 1000 mg/kg. Phytochemical analysis study showed that extract is positive for carotenoids, flavonoids, phenols and negative for alkaloids. Pre-treatment with extract prevented lipid peroxidation and showed significant increase antioxidant enzyme activity. Phytotoxic study showed that no change in biochemical and haematological, gross and histopathological changes noticed, hence no observed adverse effects of *F. virens* in rats was determined at dose 1000 mg/kg.

Keywords: *Ficus virens*; phytotoxic; phytochemical analysis; HPTLC

Introduction

India is rich source of plant kingdom and most of the plants have medicinal or toxic importance. *Ficus virens* (Syn. *Ficus infectoria*, *Ficus lacur*) is a plant belonging to the genus *Ficus* and family Moraceae (Mulberry), its common name is white fig (locally known as bilibasari) and found in India, Southeast Asia, Malaysia and Northern Australia. The trees come under genus *Ficus* have immense medicinal value as they are rich in carotenoids, total phenolic, tannins and flavonoids which shown to possess various biological properties related to antioxidant or free radical scavenging properties, such plants can be the potential candidates for the use in stress related diseases (Larson, 1988; Munganthayaran *et al.* 2007) ^[6, 9]. Among the many species, most important 4 trees with milky latex namely *Ficus racemosa*, *Ficus virens*, *Ficus religiosa*, and *Ficus benghalensis* that constitute the group "Nalpamaram" in Ayurveda and it has been reported that barks of these species have an important ingredient in many Ayurvedic formulations, such as Nalpamaradi tailam, Chandanasavam, and Saribadyasavam have lot of medicinal value (Sivarajan and Balachandran, 1994). *Ficus virens* has been reported to have *in vitro* antioxidant property (Anandjiwala *et al.*, 2008; Abdel-Hameed, 2009) ^[2, 1].

In the process of clinical investigation, a number of naturally occurring plants in western ghat regions of Karnataka showed medicinal and toxic effect to animals (Narayana *et al.* 2003) ^[10]. However, there were no any data on detail systematic protective and toxicological studies on the plant *Ficus virens*. Hence the present study was aimed to conduct protective and phytotoxic effect of a methanolic leaf extract of *Ficus virens* in a systematic way using Wistar albino rat as model.

Materials and Methods

Plant extract

Ficus virens fresh leaves were collected from Talaguppa area in Shimoga District (Karnataka state), shade dried (10 days), finely powdered and stored in air tight container until the preparation of extract. The powder (100g) was mixed in 1000 ml of methanol, kept for 5 days and were periodically shaken using an electric shaker. After 5 days, contents were filtered and

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it was further concentrated by rotary flask evaporator (Superfit India Ltd., Mumbai) at 39-40 °C. The residual methanol from the extract was evaporated after keeping the extract in a petri dish in a vacuum oven at 60 °C at the pressure of 25 psi. The extract was then weighed to calculate the yield and stored in air tight container.

Phytochemical analysis

Phytochemical analysis of the *Ficus virens* leaf extract was carried out using HPTLC technique (Wagner *et al.* 1983) [17].

Experimental Design

Animals

Wistar albino rats of either sex procured from IISc, Bangalore, were used in the present work. The animals were kept separately in polypropylene cages under well-ventilated room at temperature 36 °C and < 30% relative humidity with 12 hour light/dark cycle cages and were allowed to acclimatize to the experimental conditions for one week before the commencement of actual studies. The animals were fed with standard pellet feed and water *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA with reference No.32/LPM/IAEC/2009 for laboratory animals.

Study of protective role

Total of six groups each group contains six rats. G1 (negative control) administered with distilled water which served as vehicle for dissolving the *Ficus virens* extract. G2 as CCl₄ control (positive control). G3 as standard control, treated with Vit-C (Ascorbic acid) @ 200 mg/kg and test groups G4, G5 and G6 treated with daily doses of methanolic leaf extract of *Ficus virens* @ 50, 100 and 150 mg/kg BW respectively for 7 days (Mera and Rana, 2006). At the end of the study, oxidative stress was induced in G2 to G6 animals after 6h of the last treatment by single dose of CCl₄ @ 1 ml/kg BW in liquid paraffin (1:1) (Sundaram and Mitra, 2007) [16]. On day 8 serum samples collected for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the rats were sacrificed under xylazine and ketamine anaesthesia. Liver tissue collected for estimation of antioxidant enzyme profile and portion of liver was preserved in 10% neutral buffered formalin for histopathological evaluation.

Study of antioxidant enzyme profile and lipid peroxidation

A part of liver sample was taken, washed immediately in ice-cold saline and tissue was processed for the estimation of lipid peroxidation and antioxidant enzyme activity (superoxide dismutase, catalase and glutathione peroxidase levels).

The level of thiobarbituric acid reactive substances (TBARS) which are the indicators of lipid peroxidation was measured as per the modified method Ramanarayan *et al.* (2000) [13]. This is based on reaction of malonoldialdehyde (MDA), the breakdown product of membrane polyunsaturated fatty acids, with thiobarbituric acid (TBA) to give a brilliant pink color product. The absorbance of chloroform phase having brilliant pink color was measured at 532 nm and the values were expressed as $\mu\text{mol L}^{-1} \text{cm}^{-1}$ of MDA/g tissue. Superoxide dismutase (SOD) was determined by assay mixture consisted of 2 ml of 0.1M Tris HCl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. OD value was taken for 3 min at 420 nm and expressed as units/mg protein (Marklund and Marklund, 1974) [7]. Catalase (CAT) was

estimated by addition of 0.2 ml of homogenate and 1 ml of 30 mM H₂O₂ was added and the OD value was taken at 240 nm at an interval of 1 min for 3 min and enzyme activity was expressed as μmol of H₂O₂ decomposed per min per mg of protein (Caliborne, 1985) [5]. Glutathione peroxidase (GPx) was determined by taking OD value at 412 nm and values expressed as μM of glutathione utilized / min / mg protein (Rotruck *et al.* 1973) [14]. Total protein was determined and expressed in mg per gram of liver tissue (Bruce and Baudry, 1995) [4]. The estimation of biochemical parameters were done by using clinical chemistry analyzer - Microlab 300 (Vitalab Scientific, The Netherlands) by using commercially available diagnostic kits from Merck (Ecoline®, Merck Specialties Limited).

Study of phytotoxic effect

Selection of dose

The dose is selected on the basis of draft updated test guideline 407 for “repeated dose 28-day oral toxicity study in rats”, For selecting the doses three test groups and a control group were used, but if from the assessment of other data, no effects would be expected at a dose of 1000 mg/kg body weight per day, a limit test may be performed.

Repeated dose 28 day oral toxicity study

The study procedure was followed as per (OECD TG 407) and conducted in Wistar albino rats aged 7-9 weeks weighing 150± 10g, four groups each group with six rats of both sex separately. The G1 (control) animals were provided with distilled water which was used for dissolving the *Ficus virens* leaf extract and test groups (G2, G3 and G4) were treated with the extract 50, 200 and 800mg/kg daily for 28 days respectively. The limit test group was gavaged with dose of 1000mg/kg daily for 28 days. All the animals were observed for health condition, morbidity and mortality at least twice daily. Weekly body weight, feed intake, haemato-biochemical parameters were recorded and at the end of study all group animals were sacrificed for gross and histopathological study.

Statistical analysis

All the experimental results were expressed as Mean±SEM. Data were analyzed by analysis of variance (ANOVA), post hoc analysis was carried out by Bonferroni's Multiple Comparison Test.

Results and Discussion

In the present study, methanolic leaf extract of *Ficus virens* was found positive for flavonoids, carotenoids, phenols, bitter principle, coumarins and negative for alkaloids, anthracene derivatives. This finding is in accordance with the findings of Abdel-Hameed (2009) [1] and Babu *et al.* (2010) [3] who reported that phytochemical analysis of *Ficus* species showed the presence of flavonoids and absence of alkaloids. Serum ALT, AST and lipid peroxidation were significantly increased in CCl₄ intoxicated animals as compared to control group (Table 1). CCl₄ treatment resulted in increased level of free radicals and MDA level. Treatment with *Ficus virens* leaf extract prevented the increase in TBARS (100 and 150 mg/kg as compared to CCl₄ control. Mainly Group VI (150 mg/kg) showed significant increase in SOD catalase and glutathione peroxidase activity against CCl₄ induced oxidative stress. Liver is the major site of protein metabolism and membrane bound ATPase activity and protein thiols, which are indeed physiological free radical scavengers. Total protein in liver tissue is significantly higher in test groups as compared to

CCl₄ control. In CCl₄ treated group, liver showed inflammatory cell infiltration, vacuolar degeneration and extensive areas of necrosis. Treatment with *F. virens* leaf extract (150 mg/kg) caused reversal of these changes.

The methanolic extract of *F. virens* leaves showed significant increase in free radical scavenging activity in *in vivo* condition by using rat as model. The methanolic extract of *F. virens* leaves and bark have increased free radical scavenging activity in *in vitro* condition and as plant rich in total phenolic, tannin, flavonoids and flavonol contents which are attributed to antioxidant properties (Anandjiwala *et al.* 2008; Abdel-Hameed, 2009) [2, 1]. For the evaluation of phytotoxic

effect of *Ficus virens* methanolic leaf extract did not show any significant changes ($P>0.05$) in body weight, feed consumption, biochemical and haematological parameter. Mean while no any observable clinical sign of the toxicity, gross and histopathological lesions observed in the present study, indicating that *F. virens* leaf extract showed no observed adverse effect at the limit doses 1000mg/kg. These results were supported by Phool *et al.* (2013) [12] studied acute and sub chronic toxicity studies and pharmacological evaluation of *Ficus bengalensis L.* in rats found that the plant aqueous extract was nontoxic at 1000 mg/kg in 28 days toxicity study.

Table 1: Effect of methanolic extract of *Ficus virens* on antioxidant enzyme profile and serum liver biomarkers in Wistar albino rats (n=6)

Groups	TBARS ($\mu\text{M L}^{-1}$ cm-1 MDA /g of tissue)	SOD activity (units/mg protein)	CAT activity ($\mu\text{mol of H}_2\text{O}_2/\text{min}/\text{mg protein}$)	GPx activity (units/mg protein)	AST activity (U/L)	ALT activity (U/L)	Total protein (mg/g of liver tissue)
G1	7.96±0.34	5.09±1.09	107.91±6.05	87.11±1.45	59.13±1.59	25.78±0.83	296.67±3.88
G2	30.88±0.02***	0.96±0.15*	47.18±3.27***	31.15±1.65***	170.80±1.64***	89.12±0.71***	177.25±4.48*
G3	17.34±1.05***	5.00±0.35***	96.14±2.41***	82.56±2.45***	120.49±1.24***	54.03±0.55***	281.62±1.43***
G4	25.00±1.30*	2.33±0.14*	42.37±2.89	40.11±1.79	155.19±1.33	70.38±1.63	204.63±2.17*
G5	24.40±1.38*	3.24±0.14**	59.53±4.50	50.76±1.39	150.21±0.36	65.78±0.97	225.59±6.93**
G6	23.95±0.10**	4.28±0.26***	71.31±2.86***	53.85±1.16***	148.13±1.66***	68.73±0.21**	252.17±3.00***

Where * $P<0.05$, ** $P<0.01$, *** $P<0.001$ were considered as significant and P value >0.05 is considered as non significant

Effect of *Ficus virens* on histopathological changes in CCl₄ induced oxidative stress in Wistar albino rats

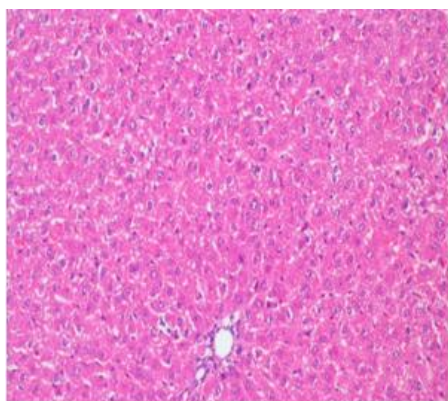


Fig 1: G1 (H and E X 100)

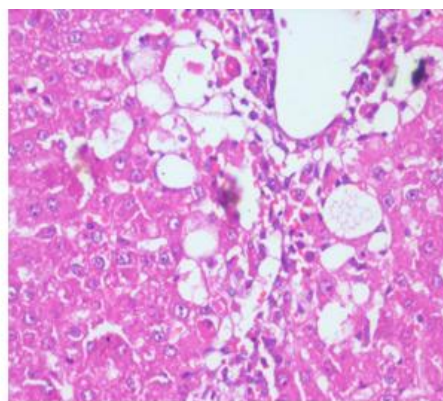


Fig 2: CCl₄ positive control, liver showing severe necrosis, vacuolar degeneration. loss of architecture (H and E X 200)

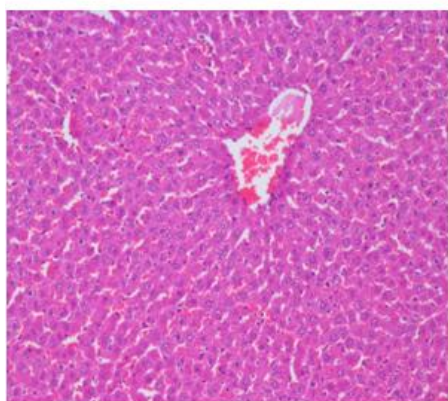


Fig 3: Vit-C treated, liver showing complete amelioration of oxidative damage(H and E X 100)

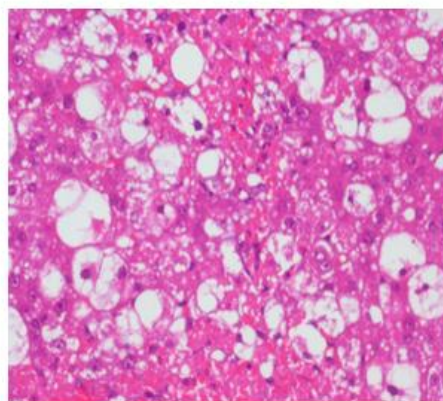


Fig 4: G4 with extensive areas of liver damage, necrosis and loss of architecture (H and E X 200)

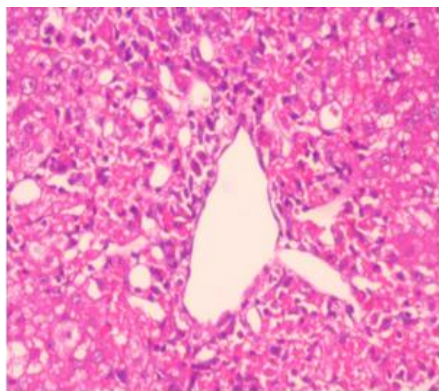


Fig 5: G5 with scare areas of necrosis, degenerative changes in liver (H and E X 200)

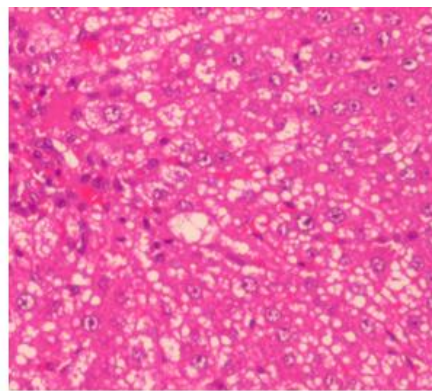


Fig 6: G6 with limited areas of necrosis in hepatocytes (H and E X 200)

Conclusion

The present study showed that methanolic leaf extract of *Ficus virens* has protective role in oxidative stress due to presence of carotenoids, flavonoids, phenols, absence of alkaloids and may have great relevance in the prevention and therapies of diseases in which oxidants or free radicals play major role. Also limit dose of extract (1000 mg/kg) intake would be tolerated for long-term use as a dietary supplementation in animals. Hence, vigorous research efforts on pharmacological properties of *F. virens* are required to use this plant in future for therapy.

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